

Effects of *Pseudomonas chlororaphis* strain AFS009 and *Beauveria bassiana* strain GHA against plumeria rust in Hawaii

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Abstract: This study examined the effects of beneficial rhizobacterium *Pseudomonas chlororaphis* strain AFS009 and entomopathogenic fungus *Beauveria bassiana* strain GHA against plumeria rust *Coleosporium plumeriae*. Two pre-emptive or three curative laboratory experiments and a curative field experiment were conducted to examine the effects of these commercially available biocontrol products. Treatments included the application of *B. bassiana* at 1.23 g/L and *P. chlororaphis* at 3 or 9 g/L. Systemic fungicide azoxystrobin applied at 0.12 g/L and water were included as positive and negative controls, respectively. While its effect was insignificant in the field trial, *B. bassiana* reduced the rust pustule development in one of two pre-emptive and two of three curative laboratory trials. In contrast, *P. chlororaphis* applied at 9 g/L suppressed the number of rust pustules in both laboratory and field experiments, demonstrating its potential biological activity against plumeria rust. In the field trial, the effect of *P. chlororaphis* was observed at 14 days post-treatment, suggesting that an application interval of 14 days on infected plants can take the rust under control.

Keywords: beneficial rhizobacteria; biological control; entomopathogenic fungus; plant disease management

Plumeria (*Plumeria* spp.), a member of Apocynaceae family, is a perennial ornamental shrub found worldwide in tropical and subtropical climatic regions. This deciduous shrub is a popular ornamental that is grown for its beautiful foliage and fragrant flowers in parks and landscape establishments. In the states of Hawaii, Texas, Florida, and California, plumeria is a well-known landscape plant in residential and commercial settings. To date, eight species of *Plumeria* and *Catharanthus roseus* (L.) G. Don host plumeria rust (*Coleosporium plumeriae* Pat.) (Kakishima

et al. 2017). The fungus attacks both mature and young leaves of plumeria but typically the latter are less susceptible due to high latex content. The latex demonstrated chitinase activity and is known to inhibit germination of uredinio spores (Weeraratne & Adikaram 2006). Infection is characterized by its powdery rust pustules of bright yellow-orange urediniospores on the underside of leaves. The rust fungus is less likely to kill the plant or directly affect its flowers, but defoliation reduces its photosynthetic ability and aesthetic values of the ornamental.

Several control measures have been recommended to manage the plumeria rust. One simple method is sanitation. Planting plumeria in drier regions with good plant spacing lessens the chance of the fungus developing and spreading; less airflow and high humidity favor proliferation of this pathogen (Nelson 2009). Heritage® (a.i. azoxystrobin, Syngenta Crop Protection, Inc., USA) and Eagle 20EW® or Eagle 40WP® (a.i. mycobutanil, Dow Agro Sciences LLC, USA) are some chemical fungicides that have been registered to control the plumeria rust. However, chemical control of rust pathogens has some limitations owing to economic, environmental, and technological reasons (Moricca & Ragazzi 2008).

Pseudomonas chlororaphis is a beneficial rhizobacterium and is a well-known biological control agent. Various strains of *P. chlororaphis* have been reported to be active against multiple foliar and soil-borne plant pathogens including *Alternaria* (Jain & Pandey 2016), *Botrytis* (Kim et al. 2008; Ajouz et al. 2011), *Corynespora* (Kim et al. 2004, 2008), *Colletotrichum* (Bardas et al. 2009; Tagele et al. 2019), *Erwinia* (Spencer et al. 2003; Kim et al. 2008), *Fusarium* (Chin-A-Woeng et al. 1998; Jain & Pandey 2016; Huang et al. 2018), *Phytophthora* (Jain & Pandey 2016; Miguelez-Sierra et al. 2019), *Pseudomonas syringae* (Radtke et al. 1994), *Pythium* (Chatterton et al. 2004), *Sclerotinia* (Nandi et al. 2017), and *Stemphylium* (Tagele et al. 2019) among others. Another soil-borne beneficial organism is the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ownley et al. 2010). This entomopathogen is well documented for its effects against insect pests but has also shown some promise against plant pathogens. The entomopathogenic fungus has been reported to limit the growth of plant fungal pathogens *in vitro* or reduced diseases caused by *Pythium*, *Rhizoctonia*, and *Fusarium* in plant assays (Ownley et al. 2010). The biocontrol has also been shown to suppress pustule development of *Puccinia* on wheat (Sheroze et al. 2002).

To date, *P. chlororaphis* and *B. bassiana* as biological control agents have not been tested against plumeria rust. The hypothesis of this study was that *P. chlororaphis* and *B. bassiana* would suppress plumeria rust as preemptive and curative treatments. The objective of this study was to examine the effects of commercially available *Beauveria bassiana* strain GHA and *Pseudomonas chlororaphis* strain AFS009 against the plumeria rust.

MATERIAL AND METHODS

The hypothesis was tested in two preemptive or three curative laboratory trials and one curative field experiment on plumeria rust-free and infected leaves or plants.

Preemptive experiment. Two benchtop trials were conducted on June 22 (Trial I) and June 29 (Trial II) of 2019 to examine the effects of *Beauveria bassiana* strain GHA (BotaniGard®, BioWorks, USA) and *Pseudomonas chlororaphis* strain AFS009 (Howler™, AgBiome Inc., USA) against plumeria rust. Treatments included *B. bassiana* applied at 1.23 g/L (BB), and *P. chlororaphis* at low (PCL; 3 g/L) or high (PCH; 9 g/L) rates. Systemic fungicide azoxystrobin (Heritage®, Syngenta, USA) was applied at 0.12 g/L as a standard positive control (AZ) and distilled water as a negative control (NT). Rust-free plumeria leaves were collected and leaf discs prepared enough to fit 60 mm × 15 mm Petri dish (Figure 1A). With modifications to methodology by García-Nevárez and Hidalgo-Jamison (2019), the leaf discs were individually disinfected

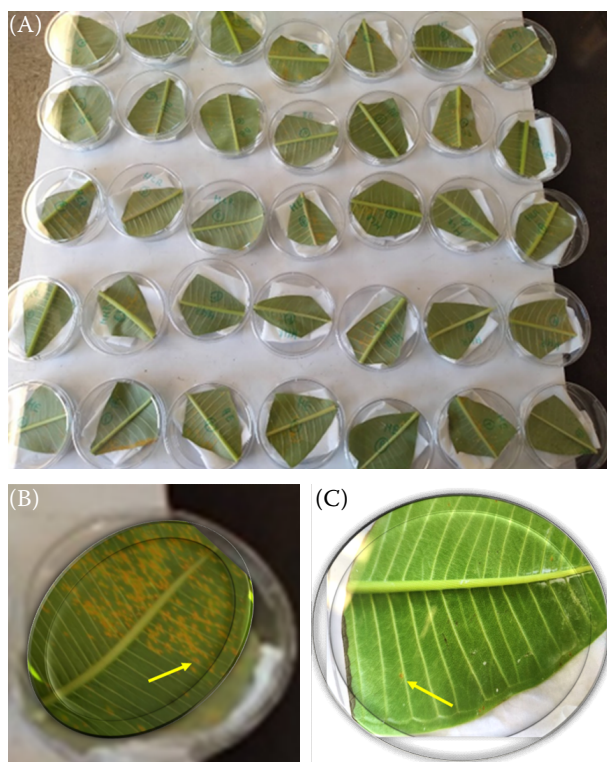


Figure 1. Laboratory experiment set up (A), untreated water control treated plumeria leaf disc (B), and *Pseudomonas chlororaphis* or azoxystrobin treated plumeria leaf disc (C)

Arrowheads are point to rust pustules

in 1% sodium hypochlorite for 10 s, double rinsed in distilled water for 10 s each time, blotted dry with a paper towel, and dipped in respective treatment suspensions for 4 hours. Then individual leaf discs were placed in Petri dishes, sprayed to runoff with plumeria rust suspension at 1.5×10^5 colony forming unit per mL, and incubated at 24 °C on the benchtop. The treatments were replicated seven times and arranged in a completely randomized design. Three observations were made at two days interval where number of rust pustules per leaf disc were counted at each time of observation (Figures 1B and 1C).

Curative experiment. Three benchtop trials were conducted on June 19 (Trial I), June 29 (Trial II) or July 8 (Trial III) to examine the effects of *P. chlororaphis* and *B. bassiana* against plumeria rust on naturally infected leaves. Infected plumeria leaves exhibiting yellow-brown pustules of the fungus were collected, leaf discs prepared in the same manner as described above, and subjected to the same fungicide treatments (NT, BB, PCL, PCH, and AZ) as in the preemptive experiments. The leaf discs were placed individually in Petri dishes and sprayed to runoff with respective treatment suspensions. Petri dishes containing the treated leaf discs were incubated at 24 °C on a benchtop. The treatments were replicated seven times, where each replicate had four Petri plates, and observed four and two times in Trials I and II, respectively. In Trial III, the treatments were replicated five times, where each replicate had four Petri plates and observed two times. All three trials were arranged in a completely randomized design. At each time of observation, the numbers of the rust pustules per cm² from four random spots on a leaf disc were counted using a 90 mm × 15 mm Petri Dish Grid (Thomas Scientific, USA).

Field experiment. A field trial was initiated on July 22, 2019, at Magoon Teaching and Research facility (21°18'24.9"N and 157°48'33.1"W) at the University of Hawaii, Honolulu, HI, where treatments were applied on selected branches of plumeria trees naturally infected with plumeria rust. Treatments included NT, BB, PCL, PCH, and AZ, similar to laboratory trials were applied at the same application rates as in the preemptive or curative experiments. In detail, five plumeria trees naturally infected with the rust fungus were selected and five branches each bearing 4–16 leaves per tree were tagged with survey tapes. Branches were each

bagged with 3.8-L plastic sampling bags to avoid spray drift. Each treatment was delivered using a hand-held Delta Plant Care Pressure Sprayer® (Delta Industries, Allentown, USA). The sprayer's nozzle was firmly held inside the sampling bag and the treatment suspensions were sprayed to runoff. Observations were made before treatment, and at 2-, 4-, 7-, 14-, 28-, and 42-days post-treatment, where counts of infected and healthy leaves per branch were recorded at each time of observation.

Statistical analysis. Data collected from the preemptive, curative, and field experiments were checked for normality using Proc Univariate in Statistical Analytical Software version 9.4 (SAS Institute Inc., USA). Where necessary, data were normalized using $\log_{10}(x + 1)$ and subjected to repeated measure analysis of variance (ANOVA) in SAS. Data within each preemptive trial and within each curative trial were combined and analyzed as no significant interactions were observed at $P \leq 0.05$ between treatment and time of observation. The data in the field experiment had interaction between treatment and time of observation, thus the data were analyzed by observation time. Only true means were presented in bar and line graphs.

RESULTS AND DISCUSSION

The significant finding in this study was that *P. chlororaphis* applied at the high rate (PCH; 9 g/L) was effective and consistent in reducing plumeria rust pustules in one preemptive (Figure 2) or three curative trials (Figure 3), and one field (Figure 4) experiments ($P \leq 0.05$). In Trial I of the preemptive experiment, PCH and *B. bassiana* applied at 1.23 g/L (BB) both suppressed the number of rust pustules similar to azoxystrobin compared to untreated (NT) water control ($P \leq 0.05$; Figure 2). In Trial II of the preemptive experiment, the number of rust pustules was too low to detect any significant differences (Figure 2). In curative trials in the laboratory, PCH reduced number of pustules on the leaf discs in all three trials while BB did so only in two of three trials; both performed in the same manner as AZ compared to NT ($P \leq 0.05$; Figure 3). In the same experiment, PCL reduced the number of plumeria rust pustules only in Trial II ($P \leq 0.05$; Figure 3). In the field experiment, PCH reduced the number of rust-infected leaves at 14-days after treatment ($P \leq 0.05$; Figure 4), a trend consistent

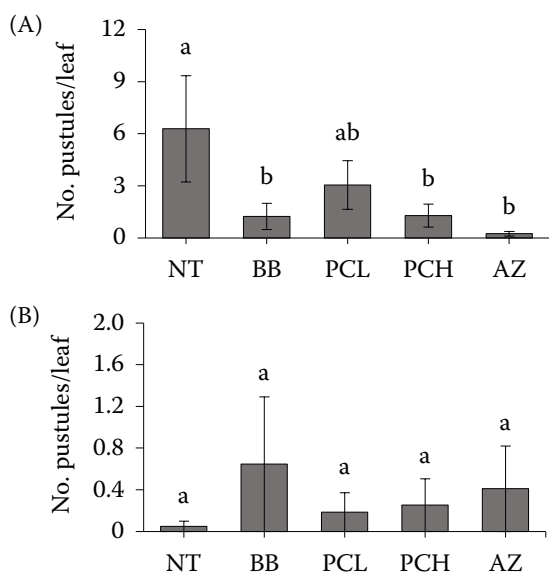


Figure 2. Means ($n = 21$) of rust pustule numbers per leaf disc affected by treatments in laboratory preemptive experiment, Trial I (A) and Trial II (B)

AZ – azoxystrobin; BB – *Beauveria bassiana*; NT – untreated water control; PCH – *Pseudomonas chlororaphis* at a high rate; PCL – *Pseudomonas chlororaphis* at a low rate
^{a,b}Means with the same letter(s) are not different at $P \leq 0.05$, according to the Waller-Duncan k -ratio ($k = 100$) t -test

with the results in preemptive and curative laboratory experiments. However, no significant treatment effect was observed at 28- and 42-days post-treatment when the experiment was terminated.

This study found that *P. chlororaphis* at the high rate (9 g/L) was effective at suppressing plumeria rust pustule development on plumeria. The effective foliar application rate is only 30% of the

minimum label rate (25.09 g/L) for ornamentals. *Pseudomonas chlororaphis* employs one or more mechanisms of action including antibiotic activity (Chin-A-Woeng et al. 1998), cell wall degrading enzymatic activity (Chin-A-Woeng et al. 1998), and elicitation or induction of host plant resistance against plant pathogens (Han et al. 2006; Kim et al. 2008). It is worth noting, in preemptive and curative experiments, that the high rate of *P. chlororaphis* suppressed plumeria rust pustule development in the same manner as azoxystrobin the systemic fungicide. This suggested that *P. chlororaphis* might have elicited not only systemic response as but also acted as an antibiotic and/or promoted enzymatic activities against the rust. Results from the field experiment, where the high rate of *P. chlororaphis* reduced the percent of infected leaves 14 days after treatment, suggested that the biological control can be applied at 14 days interval to take plumeria rust infection under control.

Beauveria bassiana is an entomopathogenic fungus that releases an array of diverse secondary metabolites including beauvericin, bassianolide, basianin, tenellin, and cyclosporin A possessing insecticidal, antibiotic, cytotoxic, and/or ionophoric properties (Logrieco et al. 2002; Keswani et al. 2013). On insect hosts, following spore attachment and germination, *B. bassiana* releases extracellular chitinases and proteases that degrade chitinous and proteinaceous components, facilitating hyphal penetration (Keswani et al. 2013). With these antibiotic, chitinolytic, and proteolytic activities, *B. bassiana* has shown promise against rust development of wheat leaf rust (Sheroze et al.

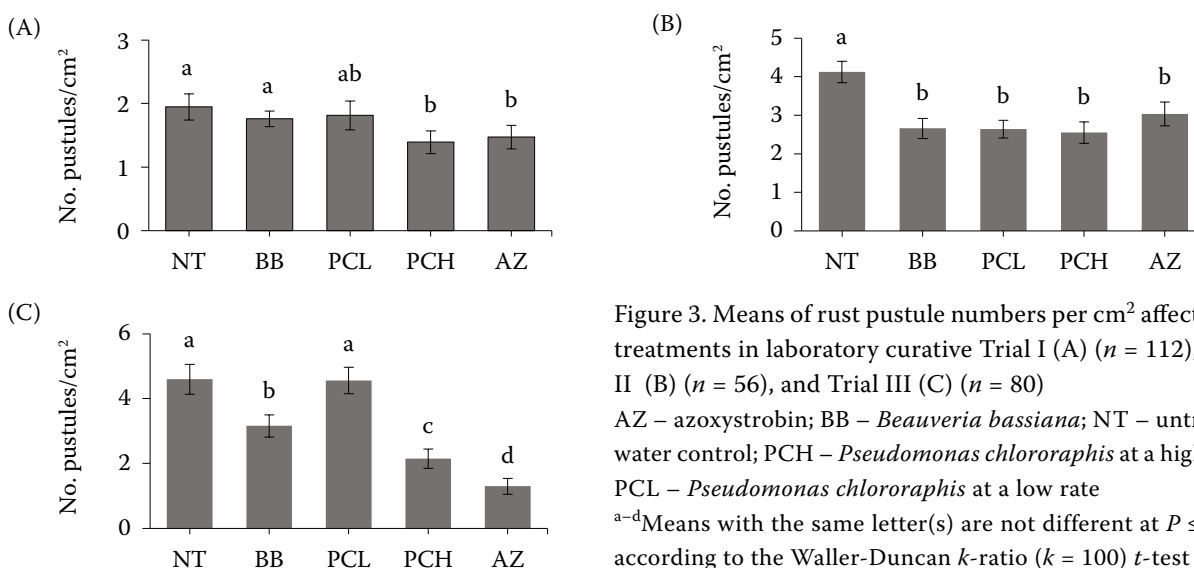


Figure 3. Means of rust pustule numbers per cm² affected by treatments in laboratory curative Trial I (A) ($n = 112$), Trial II (B) ($n = 56$), and Trial III (C) ($n = 80$)

AZ – azoxystrobin; BB – *Beauveria bassiana*; NT – untreated water control; PCH – *Pseudomonas chlororaphis* at a high rate; PCL – *Pseudomonas chlororaphis* at a low rate
^{a-d}Means with the same letter(s) are not different at $P \leq 0.05$, according to the Waller-Duncan k -ratio ($k = 100$) t -test

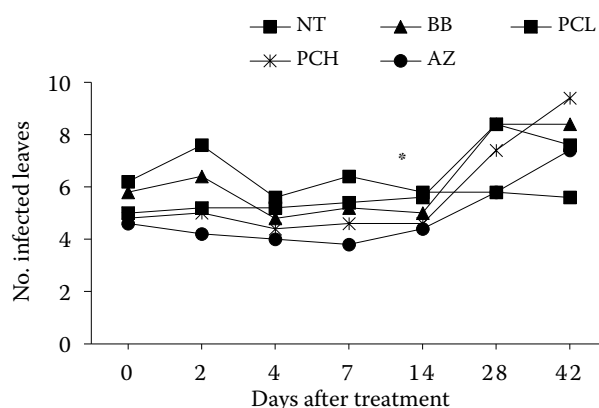


Figure 4. Effects of treatments overtime in field curative experiment

AZ – azoxystrobin; BB – *Beauveria bassiana*; NT – untreated water control; PCH – *Pseudomonas chlororaphis* at a high rate; PCL – *Pseudomonas chlororaphis* at a low rate

*Significant difference at 14 days after treatment

Lines represent means ($n = 5$)

2002). In this study, *B. bassiana* has demonstrated some activity against the plumeria rust but was not as significant and consistent as *P. chlororaphis* at 9 g/L.

Overall, *P. chlororaphis* strain AFS009 was demonstrated to be a potential biological fungicide against the plumeria rust pathogen. In fields infested with plumeria rust, foliar applications of *P. chlororaphis* at 9 g/L at 14 days interval can take the rust fungus under control.

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REFERENCES

Ajouz S., Walker A.S., Fabre F., Leroux P., Nicot P.C., Bardoin M. (2011): Variability of *Botrytis cinerea* sensitivity to pyrrolnitrin, an antibiotic produced by biological control agents. *BioControl*, 56: 353–63.

Bardas G.A., Lagopodi A.L., Kadoglidou K., Tzavella-Klonari K. (2009): Biological control of three *Colletotrichum lindemuthianum* races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365. *Biological Control*, 49: 139–45.

Chatterton S., Sutton J.C., Boland G.J. (2004): Timing *Pseudomonas chlororaphis* applications to control *Pythium aphanidermatum*, *Pythium dissotocum*, and root rot in hydroponic peppers. *Biological Control*, 30: 360–73.

Chin-A-Woeng T.F., Bloemberg G.V., van der Bij A.J., van der Drift K.M., Schripsema J., Kroon B., Scheffer R.J., Keel C., Bakker P.A., Tichy H.V., de Bruijn F.J., Thomas-Oates J.E., Lugtenberg B.J. (1998): Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Molecular Plant-Microbe Interactions*, 11: 1069–77.

García-Nevárez G., Hidalgo-Jamison E. (2019): Efficacy of indigenous and commercial *Simplicillium* and *Lecanicillium* strains for controlling *Hemileia vastatrix*. *Revista Mexicana de Fitopatología*, 37: 237–50.

Han S.H., Lee S.J., Moon J.H., Park K.H., Yang K.Y., Cho B.H., Kim Y.K., Kim K.W., Lee M.C., Anderson A.J., Kim Y.C. (2006): GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *Tabaci* in tobacco. *Molecular Plant-Microbe Interactions*, 19: 924–30.

Huang R., Feng Z., Chi X., Sun X., Lu Y., Zhang B., Ge Y. (2018): Pyrrolnitrin is more essential than phenazines for *Pseudomonas chlororaphis* G05 in its suppression of *Fusarium graminearum*. *Microbiological Research*, 215: 55–64.

Jain R., Pandey A. (2016): A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. *Microbiological Research*, 190: 63–71.

Kakishima M., Ji J.X., Zhao P., Wang Q., Li Y., McKenzie E.H.C. (2017): Geographic expansion of a rust fungus on Plumeria in Pacific and Asian countries. *New Zealand Journal of Botany*, 55: 178–86.

Keswani C., Singh S.P., Singh H.B. (2013): *Beauveria bassiana*: Status, mode of action, applications and safety issues. *Bio-tech Today*, 3: 16–20.

Kim M.S., Kim Y.C., Cho B.H. (2004): Gene expression analysis in cucumber leaves primed by root colonization with *Pseudomonas chlororaphis* O6 upon challenge-inoculation with *Corynespora cassicola*. *Plant Biology*, 6: 105–8.

Kim M.S., Cho S.M., Kang E.Y., Im Y.J., Hwangbo H., Kim Y.C., Cho B.H. (2008): Galactinol is a signaling component of the induced systemic resistance caused by *Pseudomonas chlororaphis* O6 root colonization. *Molecular Plant-Microbe Interactions*, 21: 1643–53.

Logrieco A., Moretti A., Ritieni A., Caiaffa M.F., Macchia L. (2002): Beauvericin: Chemistry, biology and significance.

- In: Upadhyay R. (ed.): Advances in Microbial Toxin Research and Its Biotechnological Exploitation. New York, USA, Kluwer Academic, 23–30.
- Migueluez-Sierra Y., Acebo-Guerrero Y., El Jaziri M., Bertin P., Hernández-Rodríguez A. (2019): *Pseudomonas chlororaphis* CP07 strain reduces disease severity caused by *Phytophthora palmivora* in genotypes of *Theobroma cacao*. European Journal of Plant Pathology, 155: 1133–43.
- Moricca S., Ragazzi A. (2008): Biological and integrated means to control rust diseases. In: Ciancio A., Mukerji K.G. (eds.): Integrated Management of Diseases Caused by Fungi, Phytoplasma and Bacteria. Berlin, Germany, Springer, 303–29.
- Nandi M., Selin C., Brawerman G., Fernando W.D., de Kievit T. (2017): Hydrogen cyanide, which contributes to *Pseudomonas chlororaphis* strain PA23 biocontrol, is upregulated in the presence of glycine. Biological Control, 108: 47–54.
- Nelson S. (2009): Plumeria rust. University of Hawaii at Manoa, cooperative extension service, Plant Disease PD-61. Available at <https://www.ctahr.hawaii.edu/oc/freepubs/pdf/pd-61.pdf> (accessed Aug 9, 2022).
- Owens B.H., Gwinn K.D., Vega F.E. (2010): Endophytic fungal entomopathogens with activity against plant pathogens: Ecology and evolution. BioControl, 55: 113–28.
- Radtke C.W., Cook S., Anderson A. (1994): Factors affecting the growth antagonism of *Phanerochaete chrysosporium* by bacteria isolated from soils. Applied Microbiology and Biotechnology, 41: 274–80.
- Sheroze A., Rashid A., Nasir M.A., Shakir A.S. (2002): Evaluation of some biocontrol agents/antagonistic microbes against pustule development of leaf rust of wheat caused by *Puccinia recondita* f. sp. *Tritici* Roberge ex. Desmaz. (Erikson and Henn.) D.M. Henderson. Plant Pathology Journal, 1: 51–3.
- Spencer M., Ryu C.M., Yang K.Y., Kim Y.C., Kloepper J.W., Anderson A. (2003): Induced defenses in tobacco by *Pseudomonas chlororaphis* strain O6 involves at least the ethylene pathway. Physiological and Molecular Plant Pathology, 63: 27–34.
- Tagele S.B., Lee H.G., Kim S.W., Lee Y.S. (2019): Phenazine and 1-undecene producing *Pseudomonas chlororaphis* subsp. *aurantiaca* strain KNU17Pc1 for growth promotion and disease suppression in Korean maize cultivars. Journal of Microbiology and Biotechnology, 29: 66–78.
- Weeraratne T., Adikaram N. (2006): Biology of *Plumeria* leaf rust disease caused by *Coleosporium plumeriae*. Ceylon Journal of Science (Biological Sciences), 35: 157–62.

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